Saskatchewan

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FINAL REPORT

BOVINE TRICHOMONIASIS: PREVALENCE IN SASKATCHEWAN AND IMPROVED DIAGNOSTIC METHODS

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Final Report (excerpt of scientific work conducted in conjunction with the project entitled "Bovine Trichomoniasis: Improved Diagnostic Methods" (Project BDF018))

OBJECTIVE(S):

Please list original objectives from approved TD-2 or MII-1 and indicate if and how they were met.

1. An evaluation of the diagnostic tests presently being used including identification of parameters which limit the sensitivity and specificity of the test (for example, the length of time sample can be in transit, temperature of sample, transport media, and culture media). The optimum conditions for the *in vitro* maintenance and growth of *T. foetus* will be identified.

The current diagnostic test has been evaluated. Optimum conditions have been identified and utilized for the development of critical control points and recommended to field staff.

2. Sample collection is a critical step in obtaining sensitive and specific diagnosis of trichomoniasis, therefore, an evaluation of various sampling protocols would be undertaken to determine the efficiency of current sampling methods. Based on the findings, an improved sampling protocol would be developed, implemented and assessed, and an educational module developed for training sample-collectors in the field.

A training video has been produced.

3. Development of a technique for the specific identification of *T. foetus*. This method would be used in conjunction with/ or replacement of culture methods. Techniques utilizing specific DNA sequences or antigens for *T. foetus* (PCR or immunohistochemistry, respectively) and in developmental stage elsewhere, require evaluation , further work, and extensive field testing for validation.

A PCR diagnostic assay has been developed and evaluated. A new proposed project is aimed at validating this assay.

4. Establish, in Saskatoon, a group of several bulls which are chronically infected with *T. foetus* in order to satisfactorily address goals 1-3.

This group was established and will be maintained in the proposed project 9905.

5. Through testing of numerous animals in natural breeding situations, validate new methods resulting from this research and establish the prevalence and epidemiological pattern of trichomoniasis in areas of Canada, particularly in association with AI centres and community pastures. This information will be important in establishing the accuracy of the new methods, identifying epidemiological risk factors and developing strategies to control/ eliminate trichomoniasis within herds.

Methods have been validated with the large group of infected animals maintained as part of the [illegible]

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project.

ANTICIPATED DELIVERABLES/PRODUCTS:

Please list original deliverables/products from approved TD-2 or MII-1 and indicate if and how they were met.

- Training module on the most effective way of collecting and transporting samples for *T. foetus*.
    - training video produced
- Molecular identifier and assay system for the specific identification of *T. foetus*.
    - Suitable PCR primers identified, extraction technique developed
- Validated protocol for the diagnostic test for *T. foetus* including the components of sample collection, transport and culture media and specific identification using molecular techniques.
    - Protocol for sample collection and transport has been developed and is outlined in the training video
    - Standardized diagnostic protocol with appropriate quality assurance measures regarding the production and utilization of appropriate culture media has been developed
    - Sensitivity and specificity of diagnostic culture has been evaluated
    - Staining technique for improved identification of *T. foetus* has been developed
    - A molecular based diagnostic assay has been developed and validation will be the subject of future research
- Valid epidemiological data on the prevalence of *T. foetus* in Canada
    - Subsequent to the submission of this project other authors have published information relating to disease epidemiology of *T. foetus*
    - Prevalence data for *T. foetus* in Canada has not been collected. Reported incidence of trichomoniasis is so low that an accurate estimation of prevalence would require a sample size beyond the scope of this project.

ANTICIPATED BENEFITS/IMPACTS:

Please list original benefits/impacts from approved TD-2 or MII-1 and indicate if and how they were met.

- Improvements in the methodology and scientific validation of the methods results in a reliable diagnostic test for *T. foetus*. Many of the benefits of offering a reliable test are often difficult to quantify in financial terms and include the following:

a) the risk of liability due to either a false positive or negative result is significantly reduced,

b) scientific advisors for importing countries recognize the high level of scientific expertise and confidently support importations of Canadian semen, embryos and live cattle,

c) transfer of the information and technology to practising veterinarians and provincial diagnostic laboratories will have a positive effect upon the reproductive fitness of the domestic cattle herd and economic well-being of the Canadian beef

industry,
d) laboratory staff performing scientifically valid diagnostic tests are enthusiastic and committed resulting in high laboratory standards and staff morale and insuring the reliability of the diagnostic test.

The validated diagnostic protocol provides a scientific basis for making recommendations regarding testing protocols, risk assessments and other issues related to trade. Veterinarians will have confidence in the sample collection and handling that they are performing. Diagnostic laboratories can utilize the developed staining technique, sample handling information, and potentially the molecular based diagnostic assay to enhance their diagnostic capability for *T. foetus*.

2. A training module on optimum collection and transport of *T. foetus* samples will raise and standardize the level of expertise offered by AAFC field staff and facilitate the training of veterinarians inexperienced in *T. foetus* sample collection.

The training module has been produced and will be available for CFIA staff and practising veterinarians to aid in sample collection and handling technique. PFRA has already expressed an interest in having this video available for its personnel.

3. With a validated protocol for *T. foetus* diagnosis, accreditation of private laboratories can proceed.

Although the current diagnostic protocol is validated, future work with the molecular based diagnostic assay may yield a test that is more suited to transfer to private laboratories.

4. Risk factors associated with the spread of trichomoniasis will be identified and long-term control measures recommended which will again benefit the domestic cattle herd and the beef industry.

Risk factors identified and published by other researchers in the field and epidemiologically based disease transmission factors have been incorporated into recommendations on disease control. They have been implemented by PFRA pastures with great success in reducing disease occurrence.

SUMMARY OF MAJOR ACHIEVEMENTS:

Media standardization/longevity/preparation

Review of the literature and media trials at the laboratory have led to the development of a standardized protocol for the preparation and utilization of the transport and culture media being used at the Centre for Animal Parasitology.

Temperature preferences - shipping recommendations

Conflicting recommendations for appropriate temperatures to keep collected samples at during shipping exist in the literature. A series of experiments was designed to evaluate the

appropriate temperature range to keep collected samples at. Samples exposed to -20 C for more than one hour failed to yield a positive culture when subsequently incubated at 37 C. Samples exposed to 4 C for more than two days showed a decreased sensitivity (Bryan et al 1999). Samples kept at 10 C and 15 C did not exhibit any decrease in sensitivity when they were subsequently incubated and examined. Recommendations for handling and shipping samples collected in the field have incorporated this information

Survival of isolates in media

All samples for the CFIA *T. foetus* testing program are handled by the Centre for Animal Parasitology in Saskatoon, Saskatchewan. This necessitates shipping samples across the country and results in a delay between sample collection analysis. Since the diagnostic test relies on the growth of any organisms collected in the initial sample, it is important that samples arrive with live viable organisms. A series of experiments demonstrated that organisms can be maintained for up to 5 days in a transport media developed at the Centre for Animal Parasitology and still exhibit good growth in the diagnostic media following processing by the laboratory (Bryan et al 1999). This information has been used to develop the protocol for handling shipped samples. Samples that arrive within 5 days of collection will still be processed by the laboratory. This eases the pressure for getting samples to their destination within 1 or 2 days, although the majority of the samples arriving at the Centre for Animal Parasitology arrive within 2 days from collection.

Growth of foreign isolates in three media

Since most diagnosis of *T. foetus* infection requires collected preputial samples to be cultured to increase the numbers of organisms present to the detection limit, it is important that the culture media used will support the growth of any strains that may be encountered. The growth characteristics of 16 different isolates from separate geographic regions was evaluated in the current media being utilized in the CFIA *T. foetus* diagnostic program as well as two other culture media. All isolates exhibited good growth in each of the three media evaluated and little difference was noted between local isolates as a group and those isolates that were different continents (1 isolated from the Czech republic and 2 isolates from Australia.) Isolates reached peak growth within the time frame of the Centre for Animal Parasitology's protocol for examination of the culture for diagnosis.

Sensitivity of diagnostic culture media

It is important to evaluate a diagnostic test using field samples from animals of known infection status. The diagnostic sensitivity of a test is the proportion of infected animals for which the diagnostic test gives a positive, or infected, result. Because there can be variability in individual animals infection it is important to utilize as many animals in a trial as possible. This project was fortunate to have thirteen infected bulls available to evaluate the diagnostic sensitivity of the current diagnostic media in use in the CFIA *T. foetus* testing program and for a commercialy available culture test kit. The sensitivity of the modified Diamond's media is estimated to be 82% (95% Confidence Interval: 67 and 91%) where as that of the commercial kit is estimated to be 96 % (95% Confidence Interval: 87 and 99%). In this study the commercial kit was more sensitive than the modified Diamond's media. However, all media used was newly purchased or prepared and no evidence was collected on any changes in diagnostic sensitivity of

the test as the media neared its expiration date. These estimates enable scientifically based recommendations for the number of tests required in different situations to be made.

Sampling

The reliability of a diagnostic test depends on many factors. The initial step in the process will be the collection of an appropriate sample. *Tritrichomonas foetus* organisms are found scattered irregularly in the crypts of the preputial mucosa in bulls. Numbers collected in samples are usually below the detection limit of a direct microscopic exam and may be close to the detection limit of the culture test. It is important to maximize the number of organisms collected in a sample. A standardized sampling technique has been developed and evaluated. This technique has been compared to a technique in common practice in some parts of the world where *T. foetus* is common and no difference in sensitivity was demonstrated (Parker et al 1999). The standardized sampling technique is simple to learn and does not require specialized equipment. When applied consistently, it has yielded good diagnostic sensitivity. A training video demonstrating this technique has been produced.

Staining

In developing a reliable diagnostic test it is important to minimize both false negatives and false positives. In a population of animals being tested where the prevalence of a particular disease would be expected to be very low the issue of false positives will take on even more importance as they be more likely to occur than true positives. In the *T. foetus* testing program the gold standard for diagnosis is the culture diagnostic test. Typically, visualization of an organism with characteristic size and motion in the culture medium is enough to have a high degree of certainty that an animal is infected with *T. foetus*. However, there are flagellated protozoans present in cattle feces that it is important to be able to differentiate. Research for this project developed an improved staining technique (Lun and Gajadhar, 1999) that allows for clear visualization of important structures to aid in identifying the organisms present in a culture test. The previous staining technique provided inconsistent results.

PCR

While culture remains the gold standard for diagnosing *T. foetus* infected animals, there are some problems associated with it's use. Occasionally samples fail to grow organisms to sufficient numbers to reach the detection limit of a direct microscopic examination due to the overgrowth of bacteria or fungi in contaminated samples. The culture diagnostic test also relies on samples arriving with viable organisms present. When samples are shipped long distances or during adverse environmental conditions viability of organisms is not always maintained. A PCR diagnostic test would not be affected by these factors as it can detect organisms whether they are viable or not. A set of primers has been acquired and evaluated. These primers appear to have good sensitivity and specificity. An extraction technique has been developed that improves the sensitivity of the diagnostic PCR assay when using clinical samples. The diagnostic PCR assay can also be used on cultured clinical samples to confirm that observed organisms are *T. foetus*. All positive cultures were identified as positive and occasionally negative cultures from known positive animals were also positive when analysed with this PCR test. This will be a valuable additional tool for diagnostic laboratories to use as a confirmatory test. It may also be useful in increasing the sensitivity of the diagnostic culture test, which would provide good incentive for

reducing the number of tests required. A number of different gut flagellates have been evaluated and none of them showed cross-reaction with the PCR diagnostic assay. This is encouraging that this test will have the desired specificity to be used as a diagnostic test in the CFIA's A.I. testing program. Sensitivity should also be sufficient to be used in this program as well. A new project to validate the use of this test as a diagnostic tool has been initiated.

Training video

Development and demonstration of appropriate sample collection and shipping techniques has been identified as one of the client needs. A training video demonstrating proper sample collection and shipment procedures has been prepared. The video can be available to field veterinarians to enhance their familiarity with collection and shipment practices for the CFIA A.I. testing program.

HAS THE CLIENT NEED BEEN ADDRESSED?

What is/will be the impact of having the need addressed? When will impacts be evident?

Yes

What is/will be the impact of having the need addressed?

A standardized diagnostic protocol with appropriate quality assurance measures provides our trading partners with an assurance of diagnostic reliability. This will enhance the accessibility of the world market for our germplasm and livestock exports.

When will impacts be evident?

The standardization of the diagnostic protocol in the laboratory is already in place. Standardization of sample collection and shipping will be implemented with the distribution of the training video.

TECHNOLOGY TRANSFER PLAN:

Was the technology transferred as planned? If not, why not? If yes, who was the recipient organisation or industry? Was help required from industry for the transfer? When and how will technology be transferred. What special considerations were there (eg. Royalties, licencing intellectual property, legal, potential conflicts of interest)? How were these dealt with?

Was the technology transferred?

Yes

Who was the recipient organisation or industry?

There are three recipient organisations for technology developed in this project. Information regarding the characteristics of the transport and culture media used in the CFIA A.I. testing program has been transferred to the *T. foetus* diagnostic program at the Centre for Animal Parasitology. This information is incorporated into their diagnostic protocol. Technology relating to improving sample collection, handling and shipping to enhance the reliability of the diagnostic test would be transferred to CFIA operations and to the PFRA.

When is the anticipated transfer schedule?
Most technology has been transferred already. The training video is completed and ready for distribution.

What was the proposed transfer method?
Information has been transferred through written reports, consultations, presentations, and a training video.

What help, if any, was needed from industry?

As this is an MII- funded project, industry provided substantial support in the form of in-kind contributions.

What special considerations are there (eg. Royalties, licencing intellectual property, legal, potential conflicts of interest)? How will these be dealt with?
None as a result of this project.

OPPORTUNITIES FOR FURTHER RESEARCH:

LIST OF PUBLICATIONS, PRESENTATIONS:
(Current or in process)

1. Xu, WD., Lun, ZR., Gajadhar, A. Chromosome numbers of *Tritrichomonas foetus* and *Tritrichomonas suis*. Veterinary Parasitology 78 (1998) 247-251.

2. Bryan, L.A., Campbell, J.R., Gajadhar, A.A. Effects of temperature on *Tritrichomonas foetus* in transport, Diamond's and InPouch™ TF media. Veterinary Record. 144 (1999) 227-232.

3. Lun, ZR, Gajadahar, A. A simple and rapid method for staining *Tritrichomonas foetus* and *Trichomonas vaginalis*. J Vet Diagn Invest. 11(1999):471-474.

4. Parker, S, Campbell, J, Ribble, C, Gajadhar, A. Comparison of two sampling tools for diagnosis of *Tritrichomonas foetus* in bulls and clinical interpretation of culture results. J Am Vet Med Assoc. 215(1999):231-235.

5. Lun, ZR, Parker, S, Gajadhar, A. Comparison of growth rates of *Tritrichomonas foetus* isolates from various geographic regions using three different culture media. Vet Parasitol (Vet Parasitology, accepted)

6. Falk, G, Parker, S, Gajadhar, A. Viability of *Tritrichomonas foetus* isolates in transport media at low temperatures. (in preparation)

7. Parker, S, Campbell, J, Ribble, C, Gajadhar, A. Differentiating the effect of sampling

sequence from sampling side on the sensitivity of the diagnostic test for *Tritrichomonas foetus* (in preparation)

8. Parker, S, Campbell, J, Gajadhar, A. Diagnostic sensitivity of two culture media for *Tritrichomonas foetus*. (in preparation)

9. Parker, S, Lun, ZR, Gajadhar, A. Utilization of a PCR-based diagnostic test for confirmation of *Tritrichomonas foetus* present in culture media. (in preparation)

LIST OF PRESENTATIONS:

1. *Tritrichomonas foetus* project progress presentation to industry. July 1997, Centre for Animal Parasitology, Health of Animals Laboratory, Saskatoon.

2. Bryan, L., Allen, J., Campbell, J., Gajadhar, A. Transport and culture conditions affecting the sensitivity diagnostic test for *Tritrichomonas foetus*. 42nd Annual Meeting, American Association of Veterinary Parasitologists, Reno, Nevada, July 19-22, 1997.

3. Parker, S., Campbell, J., Cook, H., Gajadhar, A. Diagnostic strategies for *Tritrichomonas foetus* in bulls. 42nd Annual Meeting, American Association of Veterinary Parasitologists, Reno, Nevada, July 19-22, 1997.

4. Parker, S. "Is there a better test for 'trich' ?" Presentation 2nd Annual Saskatoon Stockpersons School. Saskatoon, Saskatchewan, 1998.

5. Lun ZR and Gajadhar, A. A sensitive PCR assay for the detection of *Tritrichomonas foetus* in cattle.43rd Annual Meeting, American Association of Veterinary Parasitologists, Baltimore, Maryland, July 25-28, 1998.

6. Parker, S, Lun ZR, Falk, G, Campbell, J, and Gajadhar, A. Assessment of culture and PCR Assay for the implementation of a reliable diagnostic method for bovine trichomoniasis. 43rd Annual Meeting, American Association of Veterinary Parasitologists, Baltimore, Maryland, July 25-28, 1998.

7. Falk, G, Lun, ZR, Gajadhar, A. Viability of *Tritrichomonas foetus* field isolates in transport and culture media at various temperatures. 43rd Annual Meeting, American Association of Veterinary Parasitologists, Baltimore, Maryland, July 25-28, 1998.

8. Gajadhar A, Lun Z, Parker S, Falk G, Campbell J. Development of effective and efficient procedures for the reliable diagnosis of bovine trichomoniasis. IXTH International Congress of Parasitology, Chiba, Japan, Aug 24-28, 1998.

9. Parker, S. "Progress Report - Development of reliable methods for the diagnosis of *Tritrichomonas foetus*". Presentation to Annual Meeting PFRA District Managers and Pasture Advisory Committee. Regina, Saskatchewan, March, 1999.

10. Parker, S, Lun ZR, Gajadhar, A. Use of PCR as an ancillary test in the diagnosis of *Tritrichomonas foetus*. 44th Annual Meeting, American Association of Veterinary Parasitologists, New Orleans, Louisiana, July 10-13, 1999.